Y302, L307, F343, F403, H405, H406, D407, L427, I428, D430, G433, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2); wherein said variant has alpha-amylase activity.

213. (Amended) A variant of a parent alpha-amylase, said variant having an amino acid sequence which differs from the amino acid sequence of said parent, wherein the difference between said variant and said parent comprises a different amino acid residue in said variant than in said parent at one or more positions selected from the group consisting of the positions which correspond to amino acid residues Q298, G299, G301, Y302, L307, H405, H406, D407, I428, D430, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2); wherein said variant has alpha-amylase activity.

REMARKS

Reconsideration of the above-identified patent application is respectfully requested. In the application, claims 77-81, 84-88, 90-93, 113-187, and 192-213 are currently pending and at issue. Claims 77-81, 84-88, 90-93 and 97 have been amended to correct various informalities. Claims 120 and 135 have been amended to correct the K406 residue to H406. Support for this amendment is on page 24, line 16. Claims 135 and 165 have been amended to clarify further the claim language. Claims 193 and 213 have been amended include that the variant has alpha-amylase activity. Support for this amendment is on page 23, lines 19-21. Claims 119, 127, 134, 164 and

172 have been amended to remove V128 and H133 substitutions. The specification has been amended to incorporate a substitute sequence listing. The specification and claims have been amended to include sequence identifiers for noted sequences. Support for these amendments is in the previously filed and substitute Sequence Listing. No new matter has been added by these amendments.

INFORMATION DISCLOSURE STATEMENT:

The Examiner contends that the application does not contain an IDS.

In response, the Applicants submit an IDS concurrently herewith.

SPECIFICATION:

The Examiner contends that the application fails to comply with the requirements of 37CFR 1.821 to 1.825.

Pursuant to the requirements of 37 C.F.R. §1.821 through §1.825 for Sequence Listings, a substitute computer readable form (diskette) and a substitute paper copy containing the Sequence Listing are enclosed.

Statement Pursuant to 37 C.F.R. § 1.821:

Enclosed herewith is a computer readable form (diskette) and a paper copy containing sequence disclosure as requested by the Examiner. Pursuant to Rule 821, applicants herein state that the contents of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labeled diskette,

specifically the ASCII-encoded file therein labeled "Seqlist.txt", are identical and that the sequence submission contains no new matter.

CLAIM OBJECTIONS:

Claims 77-81, 84-88, 90-93 and 97 have been objected to for missing the word "and". Claims 78, 80, 81, and 90 also have been objected to for misspelling the word "alter".

The claims have been amended to include the word "and" to correct the spelling of "alter" as noted by the Examiner. Accordingly, Applicants request that the objection be withdrawn.

35 USC 112 REJECTIONS:

Claims 85, 93, 113, 118, 119, 126-128, 133, 134, 141, 142, 163-165, 171, 172, 179, 186, 193, 200 and 207 have been rejected for containing subject matter which is not described in the specification in such a way as to reasonably convey to one of ordinary skill in the art that the applicant had possession of the claimed invention. The Examiner contends that the claims encompass mutations in positions F403, G433, M15, N188, M197, and/or K436 that are not adequately supported in the specification. Applicants respectfully request reconsideration.

The Applicants note in the specification mutants of amino acid residues in *B. licheniformis* alpha-amylase in sequences 13-18, I428-A435, F403-V409, and D180-D204 (see page 39 lines 18-25 and page 40, lines 1-10).

This description clearly identifies to one of ordinary skill in the art that any of the amino acids within these narrow ranges may be substituted. Additionally, it is noted that one of the claimed mutations (F403) is recited as an endpoint of a disclosed range (F403-V409). One of ordinary skill in the art would be able to identify these specific mutations without undue experimentation based on the guidance from the specification. Therefore, there is sufficient written description in the specification to convey that the Applicants had substitutions as these positions and thus possession of the invention. Accordingly, Applicants request the rejection be withdrawn.

Claims 193-213 have been rejected for containing subject matter which is not described in the specification in such a way as to reasonably convey to one of ordinary skill in the art that the applicant had possession of the claimed invention. The Examiner contends that the specification does not contain a disclosure of all the potential variants that are encompassed by the claim. Applicants respectfully request reconsideration.

The claims have been amended to include that the variant protein exhibits alpha-amylase activity. Support for this amendment is on page 23, lines 19-21. The inclusion of this limitation provides one of ordinary skill in the art with the attributes and features of all of the species that are claimed within the claimed genus. Accordingly, Applicants request withdrawal of the rejection.

Claims 193-213 have been rejected for not providing reasonable enablement for any variant of an alpha-amylase comprising the mutations that does not

have functional and structural limitations. The Examiner contends that the specification is enabling for an amylase having at least one mutation listed in claim 193. The Examiner further contends that any combination of the listed residues can lead to changes of the properties of the parent amylase. The Examiner alleges that the state of the art does not allow for predictable evaluation of the function of a protein based on its structure. Applicants request reconsideration.

The claims have been amended to require that the variant protein exhibits alpha-amylase activity. Support for this amendment is on page 23, lines 19-21. Applicants request withdrawal of the rejection.

Claims 120, 135, and 165 have been rejected as being indefinite. The Examiner contends that the residue K406 is not present in SEQ ID NO:2 as claimed in claims 120 and 135. The Examiner further contends that claims 135 and 165 recite variants that "further comprise" substitutions G301 and H405, however these mutations are already recited in respective base claims. Applicants request reconsideration.

Claims 120 and 135 have been amended to change residue K406 to H406. Support for this residue can be found throughout the specifications, for example page 24, line 16. Claims 135 and 165 have been amended in matters of formal claim language. In view of these amendments, the Applicants request withdrawal of the rejection.

35 USC 102 REJECTIONS:

Claims 128-162, 166-170, and 173-187 have been rejected as being anticipated by Suzuki *et al.* The Examiner contends that Suzuki *et al.* teach the primary structures of Bacillus α-amylase resemble each other. Applicants request reconsideration.

Suzuki *et al.* only disclose the homology of the wild-type (parent) proteins. There is no discussion in Suzuki *et al.* about a variant protein, as claimed in the presently pending claims, that comprise the claimed variants. Therefore, the reference does not anticipate the present claims. Accordingly, Applicants request that the rejection be withdrawn.

Claims 118, 119, 127, 133, 134, 163, 164, 171, and 172 have been rejected as being anticipated by Bott *et al.* The Examiner contends that substitutions V128, H133, and A209 are entitled to a priority date of the parent case, which is July 18, 1996. The Examiner contends that Bott *et al.* teaches mutants the comprise the same substitutions as in the above mentioned claims. Applicants request reconsideration.

The V128 and H133 substitutions have been deleted from the claims.

The substitution of A209 was previously disclosed in the grandparent case (serial number 08/600,908; U.S. Patent Number 5,989,169). See the column 17, line 13 of the patent specification. The specification specifically states that amino acids may be substituted to produce a variant amylase (see column 17, lines 22-44). Additionally, this substitution pattern is disclosed in PCT WO 96/23874 (of which the '169 patent is a continuation) and the DK 1192/95 priority document (see page 30, line

17 and pages 28-33, respectively). Therefore, this indicates that the A209 substitution pattern is entitled to a priority date of at least February 13, 1996 (the filing date of the '169 patent). The priority date of the substitution is before the filing date of Bott (May 14, 1996). Thus, Bott does not anticipate the A209 substitution.

In view of the above amendments and arguments, the Applicants request that the rejection be withdrawn.

CONCLUSIONS

In view of the above arguments and amendments, Applications believe that the claims are in condition for allowance and such action is earnestly solicited.

Respectfully submitted,

Dated: February 26, 2001

Neepa Y. Choksi, Ph.D.
Registration No. P-47,488

EXPRESS MAIL CERTIFICATE

Date 2/26/01 Label No. EL706721535US

I hereby certify that, on the date indicated above, this paper or fee was deposited with the U.S. Postal Service & that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, DC 20231 by "Express Mail Post Office to Addressee" service.

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Docket No.:0776/1F216-US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Svendsen, *et al.*

Serial No: 09/327,563 Examiner: E. Slobodyansky

Filed: June 8, 1999 Group Art Unit: 1652

For: Alpha Amylase Mutants

MARK-UP FOR AMENDMENT OF FEBRUARY 26, 2001
PURSUANT TO 37 C.F.R. § 1.121

Hon. Commissioner of Patents
Washington, DC 20231

IN THE SPECIFICATION:

Page 8, second full paragraph:

The α- amylase structure is made up of three globular domains ordered A, B, and C with respect to sequence, which lie approximately along a line in the order B, A, C. The domains can be defined as being residues 1-103 and 206-395 for domain A, residues 104-205 for domain B, and residues 396-483 for domain C, the numbers referring to the *B. licheniformis* α-amylase (SEQ ID NO:2). This gives rise to an

elongated molecule, the longest axis being about 85 Å. The widest point perpendicular to this axis is approximately 50Å and spans the central A domain. The active site residues of the *B. licheniformis* α-amylase (SEQ ID NO: 2) are D323, D231 and E261.

Page 26, fifth full paragraph:

The calcium site of Domain C of the *B. licheniformis* α-amaylase (SEQ ID NO: 2) may be stabilized by replacing the amino acid residues H408 and/or G303 with any other amino acid residue. Of particular interest is the following mutations:

H408Q,E,N,D and/or G303N,D,Q,E

which are contemplated to provide a better calcium binding or protection from calcium depletion.

Page 32, third full paragraph:

Of particular interest is a variant of a Termamyl-like α-amylase which comprises a mutation corresponding to one or more of the following mutations in the *B. licheniformis* α-amylase (SEQ ID NO:2):

F350W

F343W

Page 32, sixth full paragraph:

Of particular interest is a variant of a Termamyl-like α-amylase which comprises a mutation corresponding to one or more of the following mutations in the *B. licheniformis* α-amylase (SEQ ID NO:2):

L427F,L,W

V481,F,I,L,W

Page 33, third full paragraph to fifth full paragraph:

Especially interesting in this connection is deletion of three amino acids within the partial sequence from T369 to I377 (referring to the sequence of *B. licheniformis* α-amylase; SEQ ID NO: 2), i.e. the partial sequence: T369-K370-G371-D372-S373-Q374-R375-E376-I377 (or the corresponding partial sequence in *B. amyloliquefaciens* α-amylase; SEQ ID NO: 4). In addition to such deletions, substitution of one or more of the undeleted amino acids within the latter partial sequence may also be advantageous.

Preferable deletions of three amino acids in the partial sequence from T369 to I377 (in *B. licheniformis* α-amylase; SEQ ID NO: 2) are deletion of K370+G371+D372 (i.e. K370*+G371*+D372*) or deletion of D372+S373+Q374 (i.e. D372*+S373*+Q374*) (or equivalent deletions in the corresponding partial sequence in *B. amyloliquefaciens* α-amylase; SEQ ID NO: 4).

Another type of mutation which would appear to be of value in improving the thermostability of these α-amylases is substitution (replacement) of the entire partial amino acid sequence from T369 to I377 (referring to the sequence of *B. licheniformis*

α-amylase; SEQ ID NO: 2) with one of the following partial sequences of six amino acids (sequence numbering increasing from left to right): I-P-T-H-S-V SEQ ID NO: 14); I-P-T-H-G-V SEQ ID NO: 15); and I-P-Q-Y-N-I SEQ ID NO: 16) (or one of the same substitutions of the corresponding partial sequence in *B. amyloliquefaciens* α-amylase; SEQ ID NO:4).

IN THE CLAIMS:

77. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to Q298 in Bacillus licheniformis.

78. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to [after] alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G299 in Bacillus licheniformis.

79. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G301 in Bacillus licheniformis.

80. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to [after] alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to Y302 in Bacillus licheniformis.

81. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to [after] alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to L307 in Bacillus licheniformis.

84. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to F343 in Bacillus licheniformis.

85. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to F403 in Bacillus licheniformis.

86. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to H405 in Bacillus licheniformis.

87. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to H406 in Bacillus licheniformis.

88. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to after the characteristics of said calcium binding site and thereby after the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to D407 in Bacillus licheniformis.

90. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to [after] alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to L427 in Bacillus licheniformis.

91. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to I428 in Bacillus licheniformis.

92. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to D430 in Bacillus licheniformis.

93. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G433 in Bacillus licheniformis.

97. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G475 in Bacillus licheniformis.

113. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to one or more of Q298, G299, G301, Y302, L307, F343, F403, H405, H406, D407, L427, I428, D430, G433, and G475 in Bacillus licheniformis (SEQ ID NO:2).

sub 1 G1

118. (Amended) The alpha-amylase according to claim 113, wherein said alpha-amylase further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

sub 1 G2

119. (Amended) The alpha-amylase according to claim 113, wherein said alpha-amylase comprises a substitution or deletion at one or more residues equivalent to M15, [V128, H133,] N188, A209, and/or M197 in Bacillus licheniformis.

120. (Amended) The alpha-amylase according to claim 113 which is modified by substituting an amino acid residue at a position corresponding to one or more of G301, H405 and/or [K]H406 in Bacillus licheniformis.

121. (Amended) An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain and comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to one or more of Q298, G299, G301, Y302, L307, F343, H405, H406, D407, I428, D430, and G475 in Bacillus licheniformis (SEQ ID NO:2).

126. (Amended) The alpha-amylase according to claim 121, wherein said alpha-amylase further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

sub G3

127. (Amended) The alpha-amylase according to claim 121, wherein said alpha-amylase further comprises a substitution or deletion at one or more residues equivalent

to M15, [V128, H133,] N188, A209, and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

128. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, at least one amino acid residue at a position corresponding to one or more of, Q298, G299, G301, Y302, L307, F343, F403, H405, H406, D407, L427, I428, D430, G433, and G475 in Bacillus licheniformis (SEQ ID NO:2) has been substituted.

133. (Amended) The variant according to claim 128, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

134. (Amended) The variant according to claim 128, wherein said variant further comprises a substitution or deletion at one or more residue equivalent to M15, [V128, H133,] N188, A209, and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

135. (Amended) The variant according to claim 128, wherein said variant [further] comprises a substitution at an amino acid residue at a position corresponding to one or more of G301, H405 and/or [K]H406 in Bacillus licheniformis (SEQ ID NO:2).

136. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, at least one amino acid residue at a position corresponding to one or more of Q298, G299, G301, Y302, L307, F343, H405, H406, D407, I428, D430, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

141. (Amended) The variant according to claim 136, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2).

142. (Amended) The variant according to claim 136, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, V128, H133, N188, A209, and/or M197 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2).

143. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to Q298 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

144. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to G299 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

145. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to G301 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

146. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to Y302 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

147. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to L307 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

148. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to F343 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

149. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to F403 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

150. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to H405 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

151. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to H406 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

152. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to D407 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

153. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to L427 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

154. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to I428 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) which has been substituted.

155. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to D430 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

156. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to G433 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

157. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, or has an amino acid sequence at least 70% homologous to SEQ ID

Nos: 2, 4, 6, or 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, and

wherein, in said variant, the amino acid residue at a position corresponding to G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

158. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, at least one amino acid residue at a position corresponding to one or more of, Q298, G299, G301, Y302, L307, F343, F403, H405, H406, D407, L427, I428, D430, G433, and G475 in Bacillus licheniformis (SEQ ID NO:2) has been substituted.

163. (Amended) The variant according to claim 158, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

164. (Amended) The variant according to claim 158, wherein said variant further comprises a substitution or deletion at one or more residue equivalent to M15, [V128, H133,] N188, A209, and/or M197 in Bacillus licheniformis (SEQ ID NO:2).

165. (Amended) The variant according to claim 158, wherein said variant [further] comprises a substitution at an amino acid residue at a position corresponding to one or more of G301[,] and/or H405 [and/or K436] in Bacillus licheniformis (SEQ ID NO:2).

166. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, at least one amino acid residue at a position corresponding to one or more of Q298, G299, G301, Y302, L307, F343, H405, H406, D407, I428, D430, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

171. (Amended) The variant according to claim 166, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, N188, A209 and/or M197 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2).

172. (Amended) The variant according to claim 166, wherein said variant further comprises a substitution or deletion at one or more residues equivalent to M15, [V128, H133,] N188, A209, and/or M197 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2).

173. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to Q298 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

174. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to G299 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

175. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to G301 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

176. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to Y302 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

177. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to L307 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

178. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to F343 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

179. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to F403 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

180. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to H405 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

181. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to H406 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

182. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to D407 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

183. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to L427 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

184. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to I428 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) which has been substituted.

185. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to D430 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

186. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to G433 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

187. (Amended) A variant of a parent alpha-amylase,

wherein said parent alpha-amylase has the amino acid sequence of SEQ ID Nos: 2, 4, 6, or 13, and

wherein, in said variant, the amino acid residue at a position corresponding to G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2) has been substituted.

193. (Amended) A variant of a parent alpha-amylase, said variant having an amino acid sequence which differs from the amino acid sequence of said parent, wherein the difference between said variant and said parent comprises a different amino acid residue in said variant than in said parent at one or more positions selected from the group consisting of the positions which correspond to amino acid residues Q298, G299, G301, Y302, L307, F343, F403, H405, H406, D407, L427, I428, D430, G433, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2); wherein said variant has alpha-amylase activity.

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213. (Amended) A variant of a parent alpha-amylase, said variant having an amino acid sequence which differs from the amino acid sequence of said parent, wherein the difference between said variant and said parent comprises a different amino acid residue in said variant than in said parent at one or more positions selected from the group consisting of the positions which correspond to amino acid residues Q298, G299, G301, Y302, L307, H405, H406, D407, I428, D430, and G475 in Bacillus licheniformis alpha-amylase (SEQ ID NO:2); wherein said variant has alpha-amylase activity.

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Respectfully submitted,

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